# Supplemental Information

## Progranulin Deficiency Promotes Circuit-Specific Synaptic Pruning via Complement Activation by Microglia

Hansen Lui, Jiasheng Zhang, Stefanie R. Makinson, Michelle K. Cahill, Kevin W. Kelly, Hsin-Yi Huang, Yulei Shang, Michael C. Oldham, Lauren Herl Martens, Fuying Gao, Giovanni Coppola, Steven A. Sloan, Christine L. Hsieh, Charles C. Kim, Eileen H. Bigio, Sandra Weintraub, Marek-Marsel Mesulam, Rosa Radermakers, Ian R. Mackenzie, William W. Seeley, Anna Karydas, Bruce L. Miller, Barbara Borroni, Roberta Ghidoni, Robert V. Farese, Jr., Jeanne T. Paz, Ben A. Barres, Eric J. Huang

## Contents:

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### Animals

*Grn*$^{-/-}$ mice have been previously described (Martens et al., 2012). *C1qa*$^{-/-}$ mice were generously provided by Dr. Marina Botto at Imperial College London (Botto et al., 1998). *Grn*$^{-/-}$*;C1qa*$^{-/-}$ double knockout mice were generated by sequential mating between *Grn*$^{-/-}$ and *C1qa*$^{-/-}$ and between the compound heterozygotes. All mice were maintained in standard barrier facility at the University of California San Francisco. Animal care was approved by the Institution of Animal Care and Use Committee (IACUCU) and followed the National Institute of Health guidelines.

### Brain Region-Specific Microarray

The number of *Grn*$^{+/+}$, *Grn*$^{+/-}$ and *Grn*$^{-/-}$ mice used for the microarray analyses is outlined below.

| Age | 2 month | | | 6 month | | | 9 month | | | 12 month | | | 18 month | | | Total # |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Crb | Ctx | Hip | Crb | Ctx | Hip | Crb | Ctx | Hip | Crb | Ctx | Hip | Crb | Ctx | Hip | |
| *Grn*$^{+/+}$ | 5 | 6 | 5 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 4 | 55 |
| *Grn*$^{+/-}$ | 5 | 5 | 6 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 51 |
| *Grn*$^{-/-}$ | 5 | 6 | 5 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 4 | 55 |

Abbreviations: Crb, cerebellum; Ctx, cerebral cortex; Hip, hippocampus.

### Brain Region-Specific Microarray and Bioinformatics Analyses

Following the identification of specific gene modules that showed age-dependent up-regulation in *Grn*$^{-/-}$ mouse brain, the top 40 or 100 genes from each module were cross-referenced with cell type-specific transcriptomes from adult mouse brain (Zhang et al., 2014). These modules were further subjected to system-level functional analyses by determining the

topographical overlap (Zhang and Horvath, 2005) among their constituent genes as previously described (Oldham et al., 2008). Network plots were produced using the iGraph Package in R (http://igraph.org/r/). To create the principle component and heatmap plots, raw microarray data was quantile normalized using the normalize.quantiles function in R. If multiple probes matched an identical Entrez gene identifier, the probe with the maximum mean expression values across all samples was kept. Principle component analysis was performed on the top 500 most variable genes across all samples using the prcomp function in R. Hierarchical clustering of the top 200 most variable genes was performed with distance computed from 1-Pearson correlation between genes and clustered with the average agglomeration method using the hclust function in R. The heatmap was created using the heatplot function in R with default parameters.

**Real-Time RT-PCR**

Brains from 4, 9, 12 and 16 months old *Grn*$^{+/+}$ and *Grn*$^{-/-}$ mice were dissected to isolate RNA from cerebral cortex, hippocampus, caudate/putamen, cerebellum and thalamus. RNA, prepared from different brain regions, primary microglia and cortical neurons, was reverse transcribed using the SuperScript III First Strand Synthesis System (Invitrogen). The primers used for QPCR were as follows: *C1qa* (Forward: 5' – AAA GGC AAT CCA GGC AAT ATC A – 3' and Reverse: 5' – TGG TTC TGG TAT GGA CTC TCC – 3') and *C3* (Forward: 5' – CCA GCT CCC CAT TAG CTC TG – 3' and Reverse: 5' – GCA CTT GCC TCT TTA GGA AGT C – 3'). SYBR green master mix (Qiagen, Valencia, CA) reactions were performed on an ABI Prism 7700 (Applied Biosystems, Carlsbad, CA). The data were normalized to the internal standard and subsequently normalized to an experimental control group ($\Delta\Delta$CT method). Statistical analyses of the results were represented as mean ± SEM. Comparisons between

*Grn*$^{+/+}$ and *Grn*$^{-/-}$ samples were performed using Student's *t* test, and comparisons between two or more groups with normally distributed datasets used an ANOVA with Tukey's posthoc test.

**Isolation of Microglia via FACS**

Brain leukocytes were harvest from 4 and 16 months old *Grn*$^{+/+}$ and *Grn*$^{-/-}$ mice using previously published methods (Hsieh et al., 2013). Briefly, mouse brains were perfused, washed with ice-cold GKN buffer (8 g/L NaCl, 0.4 g/L KCl, 1.41 g/L $Na_2HPO_4$, 0.6 g/L $NaH_2PO_4$, and 2 g/L D(+) glucose, pH 7.4) and mechanistically dissociated using a 100 mm cell filter (BD Biosciences). Tissue pellets were centrifuged at 1,500 rpm, 8 min at 4$^{o}$C, resuspended in 2.5 ml NOSE buffer (4 g/L $MgCl_2$, 2.55 g/L $CaCl_2$, 3.73 g/L KCl, 8.95 g/L NaCl, pH 6-7) supplemented with 200 U/ml DNase I and 0.2 mg/ml collagenase type I, and incubated at 37$^{o}$C for 30 min to disrupt pellet and allow enzymatic reaction to permeate tissues. Leukocytes were separated on a discontinuous isotonic Percoll (90% Percoll, 10% 1.5M NaCl, GE Biosciences, Piscataway, NJ) gradient where cells were suspended in 20 mL of a 1.03 g/mL Percoll solution in GKN buffer, and underlayed with 10 mL of a Percoll solution of 1.095 g/mL in phosphate buffered saline. Cells were spun at 900 g for 20 min with no brake. The buffy layer was isolated and leukocyte analysis performed using a combination of the following antibodies: anti-CD45 (clone Ly5) allophycocyanin (eBioscience, San Diego, CA), anti-CD11b (clone M1/70) PE (Invitrogen, Grand Island, NY), or PE-Cy5 (eBioscience), anti-Ly6G (clone 1A8) PE-Cy7 (BD Biosciences). SYTOX Blue (Invitrogen) was used to gate out dead cells. Cells were sorted on a MoFlo™ XDP flow cytometer (Beckman Coulter), and data were analyzed by using FlowJo Software (Treestar, Ashland, OR, version 9.6). All data represent mean standard error of the mean (SEM).

**Primary Microglia Cultures and FACS for C1qa and C3 Expression in Microglia**

Primary microglia cultures were prepared as previously described (Martens et al., 2012). Briefly, cerebral cortices were harvested from P3-4 *Grn*$^{+/+}$ and *Grn*$^{-/-}$ pups. The meninges were removed and the cerebral cortices were dissected into smaller pieces with forceps and then triturated in DMEM, 20% FBS, Penicillin/Streptomycin (P/S), and 20 ng/ml of GM-CSF (PeproTech, Rocky Hill, NJ). The cells were grown in poly-L-lysine coated flasks. The media was replenished three days after the initial harvest. The microglia were harvested from the astrocyte layer 6-10 days later by shaking the flasks at 200 rpm for 1-2 hours at 37° C. The media was removed and the cells were pelleted. The microglia were re-suspended in Neurobasal media supplemented with N2 supplement, L-glutamine, and P/S for 18-24 hours prior to experimentation. Primary cortical neurons were prepared from E17.5 wild type C57Bl6 mice and cultured on poly-L-Lysine-coated coverslips at a density of 5 x $10^5$ cell/well.

For FACS analyses of C1qa, C3 and C3b protein expression, primary microglia with or without overnight LPS (50 ng/ml) were transferred to polystyrene tubes, blocked with 10% rat serum on ice for 10 min, and incubated with antibody for C1qa (1:300, ab71089, Abcam), C3 (1:75, AB11862, Abcam) or C3b (1:25, HM1065, Hycult) for 20 min on ice. These cells were then treated with species-specific secondary antibodies conjugated with fluorescent tags. Following incubation with antibodies, microglia were spin down and washed with 3 ml ice-cold PBS and analyzed using the BD FACSCalibur™ System (BD Biosciences, San Jose, CA).

**DQ-BSA Assays for Visualization of Proteolytic Activity in Lysosomes**

Primary microglia cultures were prepared as previously described. 7 x $10^5$ microglia/well were plated on 12-well plates in Neurobasal media supplemented with N2 supplement, L-glutamine, and P/S for 18-24 hours prior to experimentation. The cells were pulse labeled with DQ-BSA conjugated with Alexa Fluor 488 (20 µg/mL, D12050, Thermo Fisher Scientific)

or BSA conjugated with Alexa Fluor 594 (30 µg/mL, A13101, Thermo Fisher Scientific) for 30 min. Bafilomycin A1 (5 nM, B1793, Sigma) was also added to negative control groups. The DQ-BSA pulse labeling was followed by washes with PBS and a chase period (0, 1, 2, 3, 4 hours) when microglia were allowed to endocytose the DQ-BSA probes. Microglia were washed with PBS, trypsinized, and fixed with 4% PFA for 10 min. Cells were kept in cold PBS prior to FACS analysis (BD FACSCalibur). The FACS data were analyzed using FlowJo software.

**Quantification of Vesicle Size and Intensity Profile of Intracellular Organelles in Microglia**

To characterize the colocalization of PGRN with intracellular organelles, primary microglia were labeled with a combination of antibodies for PGRN (AF2557, R&D Systems), Rab5 (ab50523, Abcam), Rab7 (#9367P, Cell Signaling), Sortilin (ab16640, Abcam) or Lamp1 (#553792, BD Biosciences). Confocal images were taken with a 60X objective with a digital zoom of 3 at a resolution of 1024 dpi. For each microglia, between 3 and 5 single layer images, 0.5mm apart, were captured. Images were exported as tiff files and quantification of LAMP1, Rab7 and Sortilin were carried out using Fiji. For the quantification of LAMP1+ vesicles, Rab7+ vesicles and Sortilin+ vesicles, an average intensity threshold, with a lower limit of 34, 31 and 53, respectively, and an upper limit of 255, was applied. Using the “Analyze Particles” function in Fiji, vesicles within a single microglia were analyzed using a size threshold with a lower limit of 1 pixel and an upper limit of infinity. Additionally, a circularity constraint, of 0.25-1.0 for LAMP1+ vesicles and 0.5-1.0 for Rab7+ and Sortilin+ vesicles, was applied. This additional constraint was used to ensure that the measurements obtained were not a compilation of multiple vesicles. Particle analysis provided the number of

vesicles within the border of the microglia, as well as a measurement of the area, the circumference and Feret's diameter for each individual vesicle. Three to four single layer images from each 24 *Grn*$^{+/+}$ microglia and 24 *Grn*$^{-/-}$ from 6 distinct biological samples were used for quantification. Measurements were averaged across the 24 microglia of each group. Data were represented as mean ± SEM, and Student's *t* tests were carried out between *Grn*$^{+/+}$ and *Grn*$^{-/-}$ in Excel. In order to obtain intensity profiles for LAMP1+, Rab7+ and Sortilin+ vesicles of primary microglia, the "Show Profile" tool of the NIS-Elements AR 4.20.01 confocal software was used. A 20mm arrow was drawn through the cytoplasm, taking care to avoid the nucleus and the Golgi apparatus. A vertical auto-scale was locked at an intensity of 4,000 for all intensity profiles. Intensity profiles were exported as raster files.

**Microglia-Neuron Co-cultures and Quantification for Synaptic Density**

Microglia and cortical neuron co-cultures were established when the neurons reached 14 DIV using a slightly modified protocol (Ji et al., 2013). Cortical microglia were recovered by shaking the flasks as described above. The media was removed and the cells were washed with HBSS twice. The microglia were re-suspended in HBSS and plated onto the neurons at a 1:3 microglia to neuron ratio. Co-cultures were allowed to continue for 3 days before fixation with 4% PFA. To quantify the synaptic density around microglia in microglia-neuron co-cultures, the presence of microglia, neurons and synapse was determined by immunofluorescent staining using antibodies for Iba-1 (Wako, 019-19741), MAP2 (Abcam, ab5392), and synaptophysin (Sigma, S5768), respectively. For each co-culture coverslip, 10-15 microglia cells were chosen at random for imaging. During capture, microglia cells were centered and images were acquired using Nikon C2 Confocal Microscopy with a 60X objective. The density of synaptophysin was quantified by drawing concentric circles around

the microglia at increments of 10 μm. As control, we also quantified synaptophysin density in areas without microglia to ensure that the overall synaptic density was not affected by the presence of microglia and that the regions selected for quantification were not biased due to its location. Subsequent images were analyzed using the ModSholIAnalysis Plugin Macro (originally created as .txt file) listed below:

```
getLine(a,b,c,d,modifier);

for (i=1; i<14; i++){
        d=80;
        r= d/2;

        //makes ROIs of concentric circles
        makeOval(a-r*i,b-r*i,d*i,d*i);

        // runs Analyze Particle plugin and pulls up summary sheet
        run("Analyze Particles...", "size=10-Infinity summarize");
}

// Creates outline of circles drawn
for (i=1; i<14; i++){
        d= 80;
        r= d/2;
        drawOval(a-r*i,b-r*i,d*i,d*i);
}

    // 40 px in 10 um for 1024 image
```

Co-cultures were immunostained with antibodies for C1qa (Abcam, AB182451), CD68 (Serotec, MCA1957), and Synaptophysin (Sigma, S5768). For each co-culture coverslip, 15-20 microglia cells were chosen at random for imaging. Images were acquired using a Nikon Eclipse 90i confocal microscope with a 60X objective and 3X digital zoom using 0.5 μm steps. Subsequent images were processed by Imaris software (Bitplane, Concord, MA) to create 3D volume surface renderings of the microglia cells. The synaptophysin+ puncta in the

microglia cell were calculated by drawing an outline of the microglia cell body as determined by CD68 and utilizing Imaris' analysis functions. The synaptophysin+;C1qa+ puncta were quantified manually by surveying the extracellular periphery of the co-cultures.

**Western Blot Analyses and Synaptosome Preparation**

Protein lysates were prepared from 4, 9 and 16 months old *Grn*$^{+/+}$ and Grn$^{-/-}$ mouse brain tissues using RIPA buffer (0.1% SDS, 1% sodium deoxycholate, 1% NP-40, 20 mM Tris, pH 7.6, 150 mM NaCl, 10 mM NaF, 1 mM $Na_3VO_4$) supplemented with protease inhibitor cocktail. Proteins were separated by SDS-PAGE and transferred to PVDF membrane (Millipore). The membranes were blocked in 3% BSA before incubated with primary antibodies overnight at 4$^{o}$C. On Day 2, the membranes were washed with 0.1% TBST washing buffer followed by incubation with secondary antibodies conjugated with HRP. Primary antibodies were obtained from the following sources: C1qa (1:1,000, Barres Lab at Stanford University), C3 (1:1,000, ab11862, Abcam), Iba-1 (1:1,000, 019-19741, Wako) and actin (CP01, Calbiochem).

To provide biochemical evidence for the age-dependent accumulation of complement proteins in synapses, we isolated synaptosomes from 4, 9 and 16 months old *Grn*$^{+/+}$ and *Grn*$^{-/-}$ mouse brains as described previously (Carlin et al., 1980). Briefly, mouse brains without cerebellums were homogenized in buffer A (0.32 M sucrose, 1 mM $NaHCO_3$, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 5 mM NaF, and 2 mM $Na_3VO_4$, at a ratio of 4 ml/gm of brain tissue) supplemented with protease inhibitors using a Dounce tissue grinder (Kontes glass homogenizer No 20, 12 strokes). Cell debris were removed by centrifugation at 710 g for 10 min at 4°C. The pellet was suspended with 3 strokes in solution A, and then the nuclear fraction were removed by centrifugation at 1,400 g for 10 min at 4 °C. The crude membrane fraction in the supernatant was collected by centrifugation at 13,800X g for 10 min at 4 °C.

The pellet (crude synaptosomal fraction) was suspended in solution B (0.32 M sucrose, 1 mM $NaHCO_3$, 5 mM NaF, and 2 mM $Na_3VO_4$, 3.2 ml/gm of starting tissue) with 6 strokes of homogenizer. The resulting supernatant was collected and separated on a discontinuous sucrose gradient (1.2 M, 1 M and 0.8 M) by centrifugation at 82,500X g with a Beckman SW55Ti rotor at 4 °C for 2 hr. Following centrifugation, cytosolic, synaptosomal, and mitochondrial fractions were collected from top to bottom. Equal aliquots from individual fractions were separated by SDS-PAGE and analyzed by immunoblotting with antibodies for C1qa, C3, Iba-1 and actin.

**Immunohistochemistry and Stereology Counting**

Immunohistochemical stains were performed on 40 μm free-floating sections of 4% paraformaldehyde-fixed mouse brains, and human brain samples from UCSF, Northwestern University and University of British Columbia. The immunostaining was developed using DAB technique and counterstained with Hematoxylin. Primary antibodies for immunohistochemistry included Iba-1 (1:3000, 019-19741, Wako) and C1qa (1:1000, F0254, Dako). Staining for Lamp1 required antigen retrieval treatment, with incubating tissue sections in 10 mM sodium citrate (pH 6.0) at 95°C for 15 to 20 minutes. Iba-1-positive microglia were counted using optical fractionator based unbiased method using Stereo Investigator on a PC that is attached to an Olympus BX5 microscope with a motorized XYZ stage (MBF Biosciences, Williston, VT)(Martens et al., 2012).

**Immunofluorescence Microscopy and Quantification of Synaptic Density in Ventral Thalamus**

Immunofluorescence staining was performed on 40 μm free-floating sections prepared using a Leica cryostat. The following primary antibodies were used: mouse anti-synatophysin (1:500; Sigma), rabbit anti-parvalbumin (1:2,000; Abcam), mouse anti-VGAT (1:300; Synaptic Systems), rabbit anti-VGLUT2 (1:500; Synaptic Systems), guinea pig anti-VGLUT2 (1:500; Synaptic Systems), and rabbit anti-C1qa (1:1500; Abcam). The following secondary antibodies were used: donkey anti-mouse Alexa 488, goat anti-rabbit Alexa Fluor® 488, donkey anti-rabbit Alexa Fluor® 488 (Life Technologies), goat anti-guinea pig Alexa Fluor® 568 and donkey anti-mouse Alexa Fluor® 568 (Invitrogen). DAPI was used for fluorescent nuclear counterstaining. Sections corresponding most closely to Bregma -1.70mm in the mouse brain atlas were stained. Sections were stained for VGAT to analyze pruning of inhibitory synapses, for VGLUT2 to analyze pruning of excitatory synapses, for both VGAT and C1qa to visualize C1qa tagging of inhibitory synapses, and for VGLUT2 and C1qa to examine C1qa tagging of excitatory synapses, respectively.

Confocal images were captured using a Nikon Eclipse 90i confocal microscope equipped with the NIS-Elements AR 4.20.01 software. For synaptophysin+ puncta, VGAT+ puncta and VGLUT2+ puncta, images were taken with a 60X objective with a digital zoom of 1 at a resolution of 1024 dpi. For each section, between 4 and 6 single layer images were acquired in random regions of the ventral thalamic nuclei, VPM and VPL. The z-plane with the brightest signal was chosen for image acquisition. For co-labeling of VGAT/C1qa and VGLUT2/C1qa, images were taken at 60X with a zoom of 3 at a resolution of 1024 dpi. For each animal, 3 separate regions of the ventral thalamus were randomly sampled and Z-stack images of 2 μm-steps were acquired. All images were then exported to tiff files and processed through Adobe Photoshop.

Quantification of VGAT and VGLUT2 were carried out using Fiji, a “batteries-included” distribution of ImageJ that includes many useful plugins contributed by the scientific community. For the quantification of VGAT+ puncta, an average intensity threshold, with a lower limit of 64 and an upper limit of 255, was applied. For the quantification of VGLUT2+ puncta, an average intensity threshold, with a lower limit of 81 and an upper limit of 255, was applied. Using the "Analyze Particles" function in FIJI, puncta were then analyzed within a 400 X 400 pixel box using a size threshold with a lower limit of 10 pixels and an upper limit of infinity. Particle analysis yielded the number of puncta within the delineated area and the size of each particle. Objects identified through this process were inspected in order to make sure that particles were indeed distinct puncta. For each image acquired, particle analysis was carried out on 3 random 400 X 400 pixel regions, avoiding regions where blood vessels were present. These quantifications were then averaged in order to obtain the average quantification for each animal for VGAT and VGLUT2 respectively. The average was then calculated amongst animals of the same age and genotype: $Grn^{+/+}$, $Grn^{-/-}$, $C1qa^{-/-}$, $Grn^{-/-}$;$C1qa^{-/-}$ at 2 months old, 4 months old (except $Grn^{-/-}$), 8 months old, 12 months old and 19 months old. Data were represented as mean ± SEM, and Student’s *t* tests were then carried out for each age between: $Grn^{+/+}$ and $Grn^{-/-}$, $Grn^{+/+}$ and $C1qa^{-/-}$, and $Grn^{+/+}$ and $Grn^{-/-}$;$C1qa^{-/-}$.

**Electron Microscopy (EM) and Immunogold EM**

Mouse and human brain samples for EM were fixed in 3% glutaraldehyde and 1% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) overnight. Following the fixation, the tissues were processed through 2% osmium tetroxide and 4% uranyl acetate, then dehydrated and embedded in Eponate 12 resin (Ted Pella Inc., Redding, CA). Ultra-thin sections were cut at 1-μm thick, collected on copper grids, and imaged in a Phillips Tecnai10 transmission electron microscope using FEI software.

Prior to immunogold labeling, 40 µm-thick paraformaldehyde-fixed free-floating sections were equilibrated with a cryoprotectant solution for 2 hours at room temperature and freeze-thawed to enhance the penetration of the immunoreagents. Sections were incubated in 4% blocking serum for 1 hour and then with C1qa antibody (1:500, F0254, Dako) in 1% blocking serum overnight. The sections were then washed, incubated with 0.2% BSAc and 0.2% fish gelatin (both from Electron Microscopy Sciences, Hatfield, PA) in PBS for 10 min and then with gold-conjugated IgGs (1:100, 0.8 nm in diameter; Electron Microscopy Sciences) in PBS/BSAc/gelatin at room temperature for 2 hours. After several PBS washes, sections were post-fixed in 1% glutaraldehyde for 10 minutes and washed with distilled water. The signal of the immunogold particles was increased using a silver enhancement kit (Electron Microscopy Sciences) for 20-30 minutes at 37°C. Finally, the sections were post-fixed in 0.5% osmium tetroxide for 10 min, dehydrated, and embedded in resin.

**Grooming Behaviors, Skin Lesions and Survival**

To quantify grooming behaviors, 8 and 12 months old *Grn*$^{+/+}$, *Grn*$^{-/-}$, *C1qa*$^{-/-}$ and *Grn*$^{-/-}$*;C1qa*$^{-/-}$ mice of both genders were used as previously described (McFarlane et al., 2008; Silverman et al., 2010). Mice were singly housed in a standard mouse cage (31 cm length X 16.5 cm wide X 14.5 cm high) was created for each mouse, and were acclimated to a quiet room for an hour prior to videotaping. Each mouse was given a 10 min habituation period in a clean, empty mouse cage and then video recorded for 10 min. The video recorded session was scored for cumulative time spent grooming all body regions, including face, neck, back and hind leg, by trained observers using a stop-watch. The amount of grooming behavior was quantified as percentage of time spent in grooming area.

A cohort of *Grn*$^{+/+}$, *Grn*$^{-/-}$, *C1qa*$^{-/-}$ and *Grn*$^{-/-}$*;C1qa*$^{-/-}$ mice was established to monitor skin lesion development and survival up to 600 days in their postnatal life. Once skin lesions

developed, treatment of these lesions followed standard procedures established by UCSF Laboratory Animal Resource Center (LARC). Briefly, skin lesions were treated with topical application of chlorhexidine 0.2% solution daily until they improved. Mice whose skin lesions deteriorated despite topical treatment were considered to have reached the end-stage criteria defined by IACUC guidelines and euthanized. About 15% of *Grn*$^{-/-}$ mice developed poor motor coordination, unsteady gait and ataxia, and were euthanized for reaching the end-stage criteria.

### *In Vitro* Slice Preparation

We prepared slices for electrophysiological recordings from senescent mice using the NMDG-perfusion method (Ting et al., 2014). Briefly, 12-month-old *Grn*$^{+/+}$ or *Grn*$^{-/-}$ of either sex were deeply anesthetized with isoflurane and perfused transcardially with ice-cold NMDG solution (93 mM NMDG, 2.5 mM KCl, 1.2 mM $NaH_2PO_4$, 30 mM $NaHCO_3$, 20 mM HEPES, 25 mM glucose, 2 mM thiourea, 5 mM L-ascorbic acid, 3 mM Na-pyruvate, 12 mM N-acetyl-L-cysteine, 0.5 mM $CaCl_2 \cdot 4H_2O$ and 10 mM $MgSO_4 \cdot 7H_2O$, titrated with HCl to pH 7.4, 300 – 310 mOsm, and equilibrated with 95% $O_2$ and 5% $CO_2$). Following perfusion, mice were rapidly decapitated, and brains were removed for rapid immersion in ice-cold NMDG solution. We prepared 400 µm-thick horizontal thalamic slices containing ventrobasal thalamus and the thalamic reticular nucleus (TRN) with a Leica VT1200 microtome (Leica Microsystems). We then incubated the brain slices in a chamber with warmed NMDG solution (32°C) for 10 minutes and transferred them to a separate chamber for 1 hour incubation at 24-26°C with modified HEPES Holding artificial cerebrospinal fluid (aCSF) solution containing 92 mM NaCl, 2.5 mM KCl, 1.2 mM $NaH_2PO_4$, 30 mM $NaHCO_3$, 20 mM HEPES, 25 mM glucose, 2 mM thiourea, 5 mM L-ascorbic acid, 3 mM Na-pyruvate, 12 mM N-acetyl-L-cysteine, 2 mM $CaCl_2 \cdot 4H_2O$ and 2 mM $MgSO_4 \cdot 7H_2O$, pH 7.4, 300 – 310 mOsm, and equilibrated with 95%

$O_2$ and 5% $CO_2$. We then transferred slices to incubate in the recording aCSF solution for 30 minutes at 24-26°C, containing 126 mM NaCl, 2.5 mM KCl, 1.25 mM $NaH_2PO_4$, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 26 mM $NaHCO_3$ and 10 mM glucose, supplemented with 0.3 M L-glutamine, pH 7.4, 300 – 310 mOsm, and equilibrated with 95% $O_2$ and 5% $CO_2$.

**Multiunit Array Recording for Thalamic Oscillations**

We performed multiunit array extracellular recordings in freshly prepared horizontal brain slices (400 µm) from 12 months old *Grn*$^{+/+}$ amd *Grn*$^{-/-}$ mice that preserve intrathalamic connectivity between TRN and somatosensory thalamus as previously described (Paz et al., 2011). Briefly, we placed thalamic slices in an interface chamber at 34 °C and superfused them at a rate of 2 ml/min with carbogenated aCSF containing 126 mM NaCl, 2.5 mM KCl, 1.25 mM $NaH_2PO_4$, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 26 mM $NaHCO_3$ and 10 mM glucose, equilibrated with 95% $O_2$ and 5% $CO_2$, pH 7.4, supplemented with 0.3 mM glutamine. Extracellular multiunit recordings were carried out using silicon linear array probes (Neuronexus) to sample the entire thalamus. Signals were amplified (PZ5 amplifier, Tucker Davis Technology) 10,000 times and band-pass filtered between 100 Hz and 6 kHz. We delivered electrical stimuli to the internal capsule with a concentric bipolar tungsten electrode (50–100 kΩ, FHC) to measure evoked responses. The stimuli were 100 µs in duration, 50 V in amplitude and delivered once every 30 seconds. Recordings were analyzed using custom MATLAB programs for automatic spike detection and spike frequency calculations. AP firing frequency (Hz) was calculated by pooling APs over 10 ms bins, averaging across the 8 – 10 channels that span VB thalamus (see orange box in **J** for channel location), and subsequent averaging across mice. For statistical analysis, we compared slope of the evoked response as fit by linear regression between *Grn*$^{+/+}$ and *Grn*$^{-/-}$ mice and compared the relative probability of action potential firing frequency between groups using the Kolmogorov-Smirnov

Test.

**Postmortem Brain Pathology and ELISA Assays for C1qa and C3b in CSF Samples from FTLD *GRN* Carriers**

All FTLD patients with *GRN* mutations used in this study have received extensive clinical neurological and neuropsychological evaluation at the Memory and Aging Center (MAC) at UCSF, the Alzheimer's Disease Center (ADC) at the Northwestern University and at the University of British Columbia. Briefly, of the 16 FTLD *GRN* carriers, 11 were diagnosed with frontotemporal dementia (FTD) or behavioral variant FTD, characterized by a range of behavioral, emotional and motor symptoms, including hyperoral behaviors, stereotyped and/or repetitive behaviors, deterioration of personal hygiene, hyperactivity, hypersexuality, impulsivity, apathy, lack of insight, emotional blunting and movement disorders (Miller, 2013). Four patients were diagnosed with primary progressive aphasia (PPA) and two with dementia, not otherwise specified (NOS)(**Table S2**). The brain pathology, clinical manifestation and genetic characterization of a majority of these cases have been previously published (Baker et al., 2006; Bit-Ivan et al., 2014; Chen-Plotkin et al., 2011; Davion et al., 2007; Gass et al., 2006; Mukherjee et al., 2008). Cerebrospinal fluid (CSF) from control subjects with no known neurodegenerative diseases and patients with Grn mutations were obtained from the Memory and Aging Center (MAC) at the University of California San Francisco and Centre for Ageing Brain and Neurodegenerative Disorders and the National Centre for Alzheimer's and Mental Diseases in Italy. These subjects received mini-mental status evaluations (MMSE), a 30-point questionnaire, by board-certified neurologists to measure their cognitive impairments. C1qa and C3b levels in the CSF of control and FTLD patients with *Grn* mutations were quantified using ELISA kits (C1q ELISA [Abcam, ab170246] and C3b [Abcam, ab195461]). CSF was diluted 1:100 for the C1qa and 1:1,000 for C3b

measurements. A linear regression analysis was performed on the plotted data to determine goodness of fit ($R^2$) and significance of the linear trend. All human samples, including frontal lobe tissues and CSF, were de-identified and evaluations performed blind. Detailed demographic data, including age, gender, clinical diagnoses, MMSE, exact nature of *GRN* mutations, and neuropathology diagnoses are available in **Table S2** and **Table S3**.

## SUPPLEMENTAL REFERENCES


Baker, M., Mackenzie, I.R., Pickering-Brown, S.M., Gass, J., Rademakers, R., Lindholm, C., Snowden, J., Adamson, J., Sadovnick, A.D., Rollinson, S.*, et al.* (2006). Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature *442*, 916-919.

Bit-Ivan, E.N., Suh, E., Shim, H.S., Weintraub, S., Hyman, B.T., Arnold, S.E., McCarty-Wood, E., Van Deerlin, V.M., Schneider, J.A., Trojanowski, J.Q.*, et al.* (2014). A novel GRN mutation (GRN c.708+6_+9delTGAG) in frontotemporal lobar degeneration with TDP-43-positive inclusions: clinicopathologic report of 6 cases. J Neuropathol Exp Neurol *73*, 467-473.

Botto, M., Dell'Agnola, C., Bygrave, A.E., Thompson, E.M., Cook, H.T., Petry, F., Loos, M., Pandolfi, P.P., and Walport, M.J. (1998). Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. Nat Genet *19*, 56-59.

Carlin, R.K., Grab, D.J., Cohen, R.S., and Siekevitz, P. (1980). Isolation and characterization of postsynaptic densities from various brain regions: enrichment of different types of postsynaptic densities. J Cell Biol *86*, 831-845.

Chen-Plotkin, A.S., Martinez-Lage, M., Sleiman, P.M., Hu, W., Greene, R., Wood, E.M., Bing, S., Grossman, M., Schellenberg, G.D., Hatanpaa, K.J.*, et al.* (2011). Genetic and clinical features of progranulin-associated frontotemporal lobar degeneration. Arch Neurol *68*, 488-497.

Davion, S., Johnson, N., Weintraub, S., Mesulam, M.M., Engberg, A., Mishra, M., Baker, M., Adamson, J., Hutton, M., Rademakers, R.*, et al.* (2007). Clinicopathologic correlation in PGRN mutations. Neurology *69*, 1113-1121.

Gass, J., Cannon, A., Mackenzie, I.R., Boeve, B., Baker, M., Adamson, J., Crook, R., Melquist, S., Kuntz, K., Petersen, R.*, et al.* (2006). Mutations in progranulin are a major cause of ubiquitin-positive frontotemporal lobar degeneration. Hum Mol Genet *15*, 2988-3001.

Hsieh, C.L., Kim, C.C., Ryba, B.E., Niemi, E.C., Bando, J.K., Locksley, R.M., Liu, J., Nakamura, M.C., and Seaman, W.E. (2013). Traumatic brain injury induces macrophage subsets in the brain. Eur J Immunol *43*, 2010-2022.

Ji, K., Akgul, G., Wollmuth, L.P., and Tsirka, S.E. (2013). Microglia actively regulate the number of functional synapses. PLoS One *8*, e56293.

Martens, L.H., Zhang, J., Barmada, S.J., Zhou, P., Kamiya, S., Sun, B., Min, S.W., Gan, L., Finkbeiner, S., Huang, E.J.*, et al.* (2012). Progranulin deficiency promotes neuroinflammation and neuron loss following toxin-induced injury. J Clin Invest *122*, 3955-3959.

McFarlane, H.G., Kusek, G.K., Yang, M., Phoenix, J.L., Bolivar, V.J., and Crawley, J.N. (2008). Autism-like behavioral phenotypes in BTBR T+tf/J mice. Genes Brain Behav *7*, 152-163.

Miller, B.L. (2013). Frontotemporal Dementia, 1st edn (Oxford University Press).

Mukherjee, O., Wang, J., Gitcho, M., Chakraverty, S., Taylor-Reinwald, L., Shears, S., Kauwe, J.S., Norton, J., Levitch, D., Bigio, E.H.*, et al.* (2008). Molecular characterization of novel progranulin (GRN) mutations in frontotemporal dementia. Hum Mutat *29*, 512-521.

Oldham, M.C., Konopka, G., Iwamoto, K., Langfelder, P., Kato, T., Horvath, S., and Geschwind, D.H. (2008). Functional organization of the transcriptome in human brain. Nat Neurosci *11*, 1271-1282.

Paz, J.T., Bryant, A.S., Peng, K., Fenno, L., Yizhar, O., Frankel, W.N., Deisseroth, K., and Huguenard, J.R. (2011). A new mode of corticothalamic transmission revealed in the Gria4(-/-) model of absence epilepsy. Nat Neurosci *14*, 1167-1173.

Silverman, J.L., Tolu, S.S., Barkan, C.L., and Crawley, J.N. (2010). Repetitive self-grooming behavior in the BTBR mouse model of autism is blocked by the mGluR5 antagonist MPEP. Neuropsychopharmacology *35*, 976-989.

Ting, J.T., Daigle, T.L., Chen, Q., and Feng, G. (2014). Acute brain slice methods for adult and aging animals: application of targeted patch clamp analysis and optogenetics. Methods Mol Biol *1183*, 221-242.

Zhang, B., and Horvath, S. (2005). A general framework for weighted gene co-expression network analysis. Stat Appl Genet Mol Biol *4*, Article17.

Zhang, Y., Chen, K., Sloan, S.A., Bennett, M.L., Scholze, A.R., O'Keeffe, S., Phatnani, H.P., Guarnieri, P., Caneda, C., Ruderisch, N.*, et al.* (2014). An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci *34*, 11929-11947.